Each day plants were rapidly and gently collected from the surface of the agar from replicate containers. The plants were harvested gently to minimize glucosinolate hydrolysis by endogenous myrosinase released upon plant wounding. Samples containing approximately 40 sprouts were homogenized in 10 volumes of DMF/ACN/DMSO solvent at -50°C which dissolves nearly all the non-lignocellulosic plant material.

Harvested plants were homogenized and QR activity with and without myrosinase, was determined as described above. As can be seen in Figure 1, Phase 2 enzymeinducer potential per gram of plant is highest in seeds, but decreases gradually following germination. detectable (less than 1000 units/g) QR inducer activity was present in the absence of added myrosinase.

25

30

10

15

08997813 . 12249

## Example 4 SPROUTS HAVE HIGHER INDUCER POTENTIAL THAN MARKET STAGE PLANTS

The cruciferous sprouts of the instant invention have higher Phase 2 enzyme-inducer potential than market stage plants. More specifically, sprouts have at least a 5fold greater Phase 2 enzyme-inducing potential than mature vegetables. For example, total inducing potential 7-day-old broccoli sprouts, extracted DMF/ACN/DMSO and treated with myrosinase, as described

above, were 238,000 and 91,000 units/g fresh weight, compared to 25,000 and 20,000 units/g fresh weight for field-grown heads of broccoli cultivars Saga and DeCicco, respectively.

Sprout extracts of over 40 different members of the Cruciferae have now been bloassayed and broccoli sprouts remain the most Phase 2 enzyme-inducer-rich plants Total inducing potential of organic solvent extracts of market stage and sprout stage broccoli and daikon is shown in Table 2.

15

20

25

Zohdat. Et8Z6680

5

TABLE 2

Comparison of Inducer Potential in
Sprouts and Mature Vegetables

Activity (units/g fresh weight)			-Fold
Vegetable Cultivar*	Mature Vegetable	Sprout**	Difference
DAIKON			
Miura	625	26,316	42
Tenshun	3,333	33,333	10
Hakkai	1,471	16,667	11
Ohkura	2,857	50,000	18
BROCCOLI			
Saga	25,000	476,000	19
DeCicco	25,000	625,000	25
Everest	8,333	1,087,000	130
Emerald City	12,500	833,000	67
Packman	20,000	556,000	28

\*The commercial portion of each plant was sampled (e.g. the taproot of Raphanus sativus variety radicola [radish]), and heads of Brassicsa oleracea variety italica [broccoli]). Myrosinase was added to all extracts tested.



\*\*Broccoli sprouts were 1-day old and daikon seedlings were 4-5-days old.

10

15

20

25

30

28

Sprouts of the broccoli cultivar Everest contained 130-fold more inducer potential (units/g fresh weight) than mature vegetables. The inducer activity in broccoli was significantly higher than in daikon.

# Example 5

## INDUCER POTENTIAL OF BROCCOLI SPROUT EXTRACTS

Inducer potential of a series of water extracts of 3-day old broccoli sprouts of the cultivar Saga were Plants were prepared by first surface determined. sterilizing seeds of Brassica oleracea variety italica (broccoli) cultivar Saga by a 1 min treatment in 70% ethanol, followed by 15 min in 1.3% sodium hypochlorite containing approximately 0.001% Alconox detergent. Seeds were grown in sterile plastic containers at a density of approximately 8 seeds/cm2 for 72 hours on a 0.7% agar support that did not contain added nutrients. environment was carefully controlled with broad spectrum fluorescent lighting, humidity and temperature control (16 hours light, 25°C / 8 hours dark, 20°C).

Plants were rapidly and gently collected from the surface of the agar to minimize glucosinolate hydrolysis by endogenous myrosinase released upon plant wounding. Sprouts (approximately 25 mg fresh wt/sprout) were gently harvested and immediately and rapidly plunged into approximately 3 volumes of boiling water in order to inactivate endogenous myrosinase as well as to extract glucosinclates and isothiocyanates from the plant tissue. Water was returned to a boil and maintained at a rolling boil for 3 min. The sprouts were then either strained from the boiled infusion [tea, soup] or homogenized in it, and the residue then removed by filtration or centrifugation.

Data in Table 3 represent both homogenates and Preparations were stored at -20°C until assayed. Inducer potential of plant extracts, prepared

as described above, was determined as described in Definitions section above.

TABLE 3 Inducer Potentials of Hot Water Extracts of 3-Day Saga Broccoli Sprouts

ACTURE ETRAGGED

15

10

20

25

1 2 3 4 5 6 7 8 9	500,000 370,000 455,000 333,000 435,000 333,000 625,000
3 4 5 6 7 8 9	455,000 333,000 435,000 333,000 625,000
4 5 6 7 8 9	333,000 435,000 333,000 625,000
5 6 7 8 9	435,000 333,000 625,000
6 7 8 9	333,000 625,000
7 8 9 10	625,000
9	
9	
10	250,000
	313,000
1.1	357,000
11	370,000
12	370,000
13	217,000
14	222,000
15	1,000,000
16	714,000
17	435,000
18	1,250,000
19	263,000
AVERAGE	

Some variability in the amount of Phase 2 enzyme-inducer potential was detected. High levels of Phase 2 enzyme-inducer potential, however, were consistently observed.

5

10

15

20

# Example 6 HOT WATER BROCCOLI EXTRACTS TREATED WITH DAIKON MYROSINASE

QR activity in a hot water broccoli extract increased in the presence of a vegetable source of myrosinase. An aqueous extraction of 3-day old sprouts of broccoli cultivar Saga grown on water agar, in which myrosinase was inactivated by boiling for 3 min, was divided into 6 different 150 ml aliquots. Nine-day old daikon sprouts, a rich source of the enzyme myrosinase, were added to this cooled infusion in amounts equivalent to 0, 5, 9, 17, 29 and 40% (w/w) of the broccoli. QR activity, as determined in the Definition section, of the control extracts containing 0% daikon was 26,300 units/gram fresh weight while QR activity of the extracts that had received daikon as a source of myrosinase ranged from 500,000 to 833,000 units/gram fresh weight of broccoli. Accordingly, myrosinase present in the daikon sprouts, increased the QR activity in the broccoli extract greater than 19-fold.

25

## Example 7

# GLUCORAPHANIN AND GLUCOERUCIN ARE THE PREDOMINANT GLUCOSINOLATES IN HOT WATER EXTRACTS OF BROCCOLI (CULTIVAR SAGA) SPROUTS

Paired Ion Chromatography (PTC). Centrifuged hot water extracts of 3-day-old broccoli (cultivar Saga) sprouts were subjected to analytical and preparative PIC on a reverse phase C18 Partisil ODS-2 HPLC column in ACN/H<sub>2</sub>O (1/1, by vol.) with tetraoctylammonium (TOA) bromide as the counter-ion. Only three well-separated peaks were detected: peak A sluted at 5.5 min, B at 11.5

31

15

20

25

30

35

31

min, and C at 13 min at a molar ratio [A:B:C] of ca. 2.5 : 1.6 : 1.0 (monitored by UV absorption at 235 nm), and they disappeared if the initial extracts were first treated with highly purified myrosinase. Peaks A, B, and C contained no significant inducer activity, and cyclocondensation assay of myrosinase hydrolysates showed that only Peaks A and C produced significant quantities of isothiocyanates, accounting for all the inducer activity. See Zhang et al., Anal. Biochem. 205: 100-107 (1992). Peak B was not further characterized. Peaks A and C were eluted from HPLC as TOA salts but required conversion to ammonium salts for successful mass spectroscopy, NMR and bioassay. The pure peak materials were dried in a vacuum centrifuge, redissolved in aqueous 20 mM NH,Cl, and extracted with chloroform to remove excess TOA bromide. The ammonium salts of glucosinolates remained in the aqueous phase, which was then evaporated.

Identification of Glucosinolates. The ammonium salts of Peaks A and C were characterized by mass spectrometric and NMR techniques: (a) negative ion Fast Atom Bombardment (FAB) on a thioglyerol matrix; this gave values of 436 (Peak A) and 420 (Peak C) amu for the negative molecular ions, and (b) high resolution NMR, as shown in Figure 2, provided unequivocal identification of structure. Peak A is glucoraphanin methylsulfinylbutyl glucosinolatel, and Peak C is the related closely glucoerucin [4-methythiobutyl glucosinolate]. These identifications and purity are also consistent with the inducer potencies; Peaks A and C, after myrosinase hydrolysis had potencies of 36,100 and 4,360 units/µmol, respectively, compared with reported CD values of 0.2  $\mu$ M (33,333 units/ $\mu$ mol) for sulforaphane and 2.3  $\mu M$  (2,900 units/ $\mu mol$ ) for erucin. CD values are the concentrations of a compound required to double the QR specific activity in Hepa 1c1c7 murine hepatoma cells. Since there are no other glucosinolate peaks, and the inducer activity of peak A and C account for the total inducer activity of the extracts, it is

therefore likely that in this cultivar of broccoli, there are no significant quantities of other inducers, i.e., no indole or hydroxyalkenyl glucosinolates. Further, the isolated compounds are therefore substantially pure.

5

10

15

20

25

## Example 8

## COMPARISON OF AQUEOUS AND ORGANIC SOLVENT TECHNIQUES FOR EXTRACTION OF INDUCER POTENTIAL

Plants were prepared by first surface sterilizing seeds of Brassica oleracea variety italica (broccoli) cultivar Saga, with 70% ethanol followed by 1.3% sodium hypochlorite and 0.001% alconox. The seeds were grown in sterile plastic containers at a density of approximately 8 seeds/cm2 for 72 hours on a 0.7% agar support that did not contain added nutrients. The environment was carefully controlled with broad spectrum fluorescent lighting, humidity, and temperature control (16 hours light, 25°C/8 hours dark, 20°C).

The plants were rapidly and gently collected from the surface of the agar to minimize gludosinolate hydrolysis by endogenous myrosinase released upon plant wounding. A portion of the plants was homogenized with 10 volumes of the DMF/ACN/DMSO solvent at -50°C, as described in Example 1, which dissolves nearly all the nonlignocellulosic plant material. Alternatively, the bulk of the harvested plants was plunged into 5 volumes of boiling water for 3 min to inactivate endogenous myrosinase and extract glucosinolates to isothiocyanates. The cooled mixture was homogenized, centrifuged, and the supernant fluid was stored at -20°C.

30 Inducer potential of plant extracts, prepared by the two methods described above, was determined by the microtiter plate bicassay as described above. inducer potentials in an average of 5 preparations were 702,000 (DMF/ACN/DMSO extracts) and 505,000 (aqueous extracts) units/g fresh weight of sprouts.



1.0

15

20

25

30

Spectrophotometric quantitation cyclocondensation product of the isothiocyanates with 1,2-benzenedithiole was carried out as described in Zhang et al., Anal. Biochem. 205: 100-107 (1992). Glucosinolates were rapidly converted to isothiocyanates after addition of myrosinase. About 6% of the total hot water extractable material [dissolved solids] consisted of glucosinolates. These results demonstrate that (a) isothiocyanate levels in the crude plant extracts are extremely low; (b) myrosinase rapidly converts abundant glucosinolates to isothiocyanates; (c) hot water extraction releases over 70% of the inducer activity extractable with a triple solvent mixture permitting recovery of most of the biological activity in a preparation that is safe for human consumption; and (d) over 95% of the inducing potential in the intact plant is present as glucosinolates and therefore no other inducers are present in biologically significant quantities.

#### Example 9

#### DEVELOPMENTAL REGULATION OF GLUCOSINOLATE PRODUCTION

Preliminary experiments in which field grown broccoli (cultivar DeCicco) was harvested at sequential time points from the same field indicated that on a fresh weight basis, inducer potential declined from the early vegetative stage through commercial harvest, but appeared to increase at late harvest (onset of flowering). These data suggested that inducer potential might be highest in seeds. Subsequent studies have shown that when seeds of 8 broccoli cultivars were surface sterilized and grown under gnotobiotic conditions, Phase 2 enzyme-inducer potential was highest in seeds and declined progressively (on a fresh weight basis) over time throughout the first 14 days of seedling growth.

Expressed on a per plant basis, however, activity remained constant over this period, suggesting that at

15

20

25

30

this early stage of growth there was no net synthesis of glucosinolates. However, when the glucosinolate profiles of market stage broccoli heads and 3 day old sprouts (cultivar Emperor) were compared, there was a profound difference in the apparent glucosinolate compositions of these plants.

Sprouts were prepared by first surface sterilizing seeds of Brassica oleracea variety italica (broccoli) cultivar Emperor with a 1 minute treatment in 70% ethanol, followed by 15 min in 1.3% sodium hypochlorite with approximately 0.001% Alconox detergent. Seeds were grown in sterile plastic containers at a density of approximately 8 seeds/cm2 for 72 hours on a 0.7% agar support that did not contain added nutrients. environment was carefully controlled; broad spectrum fluorescent lighting, humidity and temperature control (16 hours light, 25°C / 8 hours dark, 20°C).

Plants were rapidly and gently collected from the surface of the agar to minimize glucosinolate hydrolysis by endogenous myrosinase released upon plant wounding. Sprouts [approximately 25 mg fresh wt/sprout], were gently harvested and immediately and rapidly plunged into approximately 3 volumes of boiling water in order to inactivate endogenous myrosinase as well as to extract glucosinolates and isothiocyanates from the plant tissue. Water was returned to a boil and maintained at a rolling boil for 3 min. The sprouts were then strained from the boiled infusion [tea, soup] and the infusion was stored at -20°C until assayed.

Market stage heads were obtained by germinating seeds of the same seedlot in a greenhouse in potting soil, transplanting to an organically managed field in Garrett County, MD and harvested at market stage. Heads were immediately frozen upon harvest, transported to the laboratory on ice and extracts were prepared in an identical fashion to those described above for sprouts

except that approximately 3 gram floret tissue samples were used for extraction.

Inducer potential of plant extracts, prepared as described above, was determined by the microtiter plate bioassay method as described in Example 1. Paired ion chromatography revealed two major peaks, probably glucobrassicin and neo-glucobrassicin, in extracts of market stage heads with similar retention times to glucobrassicin (indole-3-ylmethyl glucosinolate) and neoglucobrassicin (1-methoxyindole-3-ylmethyl glucosinolate). This observation is consistent with published reports on the glucosinolate composition of mature broccoli plants. However, paired chromatography under the same conditions of identically prepared extracts of 3-day-old sprouts showed absence of glucobrassicin or neo-glucobrassicin. Additionally, 3day-old sprouts of different broccoli cultivars produce different mixtures of glucosinolates. Accordingly, glucosinolate production is developmentally regulated.

20

25

30

10

15

#### Example 10

EVALUATION OF ANTICARCINGGENIC ACTIVITIES OF BROCCOLI SPROUT PREPARATIONS IN THE HUGGINS DMBA (9,10 DIMETHYL-1,2-BENZANTHRACENE) MAMMARY TUMOR MODEL

Sprouts were prepared by first surface sterilizing seeds of Brassica oleracea variety italica (broccoli) cultivar Saga with a 1 min treatment in 70% ethanol, followed by 15 min in 1.3% sodium hypochlorite with approximately 0.001% Alconox detergent. Seeds were grown in sterile plastic containers at a density of approximately 8 seeds/cm2 for 72 hours on a 0.7% agar support that did not contain added nutrients. environment was carefully controlled with broad spectrum fluorescent lighting, humidity and temperature control (16 hours light, 25°C / 8 hours dark, 20°C).

15

20

25

30

35

The plants were rapidly and gently collected from the surface of the agar to minimize glucosinolate hydrolysis by endogenous myrosinase released upon plant wounding. A large quantity of sprouts was harvested by immediately and rapidly plunging into approximately 3 volumes of boiling water in order to inactivate endogenous myrosinase, as well as extracting glucosinolates and isothiocyanates from the plant tissue. Water was returned to a boil and maintained at a rolling boil for Sprouts were then strained from the boiled infusion [tea, soup] and the infusion was lyophilized and stored as a dry powder at -20°C [designated Prep A]. Other sprouts, similarly prepared were extracted with boiling water, cooled to 25°C and were amended with a quantity of 7 day old daikon sprouts equivalent to approximately 0.5% of the original fresh weight of broccoli sprouts. This mixture was homogenized using a Brinkman Polytron Homogenizer and incubated at 37°C for 2 hours following which it was filtered through a sintered glass filter, lyophilized as above and stored as a dried powder at -20°C [designated Prep B].

QR inducer activity and inducer potential of plant extracts, prepared as described above, was determined by the microtiter plate bioassay method as described above. The induction of QR activity in preparation A is largely due to glucosinolates; predominantly glucoraphanin, which is the glucosinolate of sulforaphane, but this preparation also contains some glucoerucin, which is the sulfide analog of glucoraphanin, The induction OR activity of preparation B is almost exclusively due to isothiocyanates arising from treatment of glucosinolates with myrosinase.

Female Sprague-Dawley rats received at 35 days of age were randomized; 4 animals per plastic cage. All animals received 10 mg DMBA, by gavage in 1 ml sesame oil, at age 50 days. Sprout preparations (A or B) or vehicle control were given by gavage at 3, 2 & 1 day prior to DMBA, on

the day of DMBA (2 hr prior to the DMBA dose) and on the day following DMBA dosing. The vehicle used was 50% Emulphor 620P / 50% water. Animals were maintained on a semi-purified AIN-76A diet ad libitum from the time of receipt until termination of the experiment (167 days of age).

Zetalt. Etazesso

Cohzer" Erazosso

ANTICARCINOGENIC ACTIVITIES OF BROCCOLI SPROUT EXTRACTS IN THE DWBA RAT MAMMARY TUMOR MODEL TABLE 4

	GROUP	TREATMENT	NUMBER OF ANIMALS AT TERMINATION	TOTAL TUKOR NUMBER	MULTIPLICITY: NUMBER OF TUMORS PER RAT
ហ	CONTROL	DMBA only	19	34	1.79
٠	PREPARATION A (Glucosinolate)	324 mg/dose (100 µmol sulforaphane equiv.)	1.8	19	1.05
10	PREPARATION B (Isothiocyanate)	424 mg/dose (100 µmol sulforaphane equiv.)	20	11	0.55

Case 1:07-cv-07844-SAS

10

20

25

30

39

The development of palpable tumors was delayed for as much as 5 weeks by the administration of sprout extracts. Rats treated with either Preparation A or B had significantly fewer tumors than the untreated control, and the multiplicity of tumors (tumors per rat) was significantly lower in the animals receiving Preparations A or B.

## Example 11 METABOLISM AND CLEARANCE OF GLUCOSINOLATES IN HUMANS

Two male, non-smoking volunteers ages 35 and 40 years, each in good health, were put on a low vegetable diet in which no green or yellow vegetables, or condiments, mustard, horseradish, tomatoes or papayas were consumed. After 24 hours on such a diet, all urine was collected in 8 hr aliquots. After 24 hours of baseline data, subjects ingested 100 ml of broccoli sprout soup (prepared as below), containing 520 µmol of glucosinolates.

The sprouts were prepared by first surface sterilizing seeds of Brassica oleracea variety italica (broccoli) cultivar Saga with a 1 min treatment in 70% ethanol, followed by 15 min in 1.3% sodium hypochlorite with ca. 0.001% Alconox detergent. Seeds were grown in sterile plastic containers at a density of approximately 8 seeds/cm2 for 72 hours on a 0.7% agar support that did not contain added nutrients. The environment was carefully controlled with broad spectrum fluorescent lighting, humidity and temperature control (16 hours light, 25°C / 8 hours dark, 20°C). The plants were rapidly and gently collected from the surface of the agar to minimize glucosinolate hydrolysis by endogenous myrosinase released upon plant wounding. quantity of sprouts was harvested by immediately and rapidly plunged into approximately 3 volumes of boiling water in order to inactivate endogenous myrosinase as

well as to extract glucosinolates and isothiocyanates from the plant tissue. Water was returned to a boil and maintained at a rolling boil for 3 min. Following the boiling step, sprouts were homogenized directly in their infusion water for 1 min using a Brinkman Polytron Homogenizer and the preparations were frozen at -79°C until use.

Inducer potential of plant extracts, prepared as described above, was determined by the microtiter plate bioassay method as described above. Inducer potential is nearly all due to glucosinolates; predominantly glucoraphanin, which ÍS. the glucosinolate sulforaphane, but some glucoerucin which is the sulfide analog of glucoraphanin was also present. When converted to isothiocyanates by the addition of purified myrosinase, Phase 2 enzyme-inducing potential was 100,000 units/ml and contained 5.2 µmol of isothiocyanates per ml, as determined by the cyclocondensation reaction described in Example 7. Thus, the subjects consumed a total of 520  $\mu$ mol of glucosinolates.

Collection of 8 hour urine samples was continued for an additional 30 hours. Urinary excretion of isothiocyanate conjugates (dithiocarbamates) was monitored using the cyclocondensation reaction as described in Example 7.

41

10

15

25

Coheer. Erszebad

## TABLE 5 EXCRETION OF DITHIOCARBAMATES BY TWO SUBJECTS INGESTING 520 MICROMOLES OF GLUCOSINOLATES

EXTRACTED FROM SAGA BROCCOLI

10 15

20

ZGHZZT"ETBZG680

5

TIME	CONDITION	SUBJECT 1	SUBJECT 2
Collection Time (hours)		µmol Dithioca per 8 hour ur collection	
8	baseline	1.4	2.7
16	baseline	2.1	0.9
24	baseline	1.7	5.4
32	1st 8 hour post-dose	23.2	20.4
40	2nd 8 hour post-dose	9.9	36.8
48	3rd 8 hour post-dose	4.4	14.0
56	4th 8 hour post-dose	4.2	4.1
Total post-dose minus average baseline:		39.8	63.2
Total as Percent of dose:		6.7%	12.2%

The two subjects studied metabolically converted a significant fraction of the ingested glucosinolates to the isothiocyanates which were converted to cognate dithiocarbamates and measured in the urine.

# Example 12 EFFECTS OF PHYSICAL INTERVENTIONS ON SPROUT GROWTH ON PRODUCTION OF INDUCERS OF QUINOME REDUCTASE

25 Sprouts were prepared by first surface sterilizing seeds of Raphanus sativum (daikon) by a 1 minute treatment with 70% ethanol, followed by a 15 min with 1.3% sodium hypochlorite with approximately 0.001% Alconox detergent. Seeds were grown

Treated sprouts were irradiated with germicidal UV light for 0.5 hr on days 5 and 6. Treated sprouts were only half the height of the untreated controls. Plants were harvested on day 7 by rapidly and gently collecting the plants from the surface of the agar to minimize glucosinolate hydrolysis by endogenous myrosinase released upon plant wounding. Sprouts were harvested by immediate and rapid plunging into approximately 10 volumes of DMF/ACN/DMSO (1:1:1) at approximately -50°C in order to inactivate endogenous myrosinase as well as to extract glucosinolates and isothicoganates. Sprouts were immediately homogenized with a ground glass mortar and pestle and stored at -20°C.

Inducer potential of plant extracts, prepared as described above, was determined by the microtiter plate bioassay method as described above. Inducer potential of the UV-treated sprouts was over three times that of untreated controls. Treatment of sprouts with ultraviolet light therefore increased the Phase 2 enzyme-inducer potential of the plant tissue.

Although the foregoing refers to particular preferred embodiments, it will be understood that the present invention is not so limited. It will occur to those of ordinary skill in the art that various modifications may be made to the disclosed embodiments and that such modifications are intended to be within the scope of the present invention, which is defined by the following claims. All publications and patent applications mentioned in this specification are indicative of the

10

15

20

25

30

Zetuer" erazesso

level of skill of those in the art to which the invention pertains.

All publications and patent applications are herein incorporated by reference to the same extent as if each individual publication or patent application were specifically and individually indicated to be incorporated by reference in its entirety.

### What Is Claimed Is:

- 1. Cruciferous sprouts, with the exception of cabbage, cress, mustard and radish sprouts, harvested prior to the 2-leaf stage.
- The cruciferous sprouts according to claim 1, wherein said sprouts are a Brassica oleracea selected from the group of varieties consisting of acephala, alboglabra, botrytis, costata, gemmifera, gongylodes, italica, medullosa, palmifolia, ramosa, sabellica, and selensia.
- The cruciferous sprouts according to claim 2, wherein said sprouts are a Brassica oleracea variety italica.
- 4. The cruciferous shrouts according to claim 1, wherein said sprouts are a Brassica oleracea variety botrytis.
- The cruciferous sprouts according to claim 1, wherein said sprouts are a Brassida oleracea variety botrytis subvariety cauliflora.
- The cruciferous sprouts \according to claim 1, wherein said sprouts are substant ally free of Phase 1 enzyme-inducing potential.
- A non-toxic solvent extract of the cruciferous sprouts according to claim 1.
- The non-toxic solvent extract according to claim 7, wherein said solvent is water.
- The non-toxic solvent extract according to claim 8, further comprising a cruciferous vegetable comprising an active myrosinase enzyme.

Cetadr. Erached

- 10. The non-toxic solvent extract according to claim 9, wherein said cruciferous vegetable is of the genus Raphanus.
- 11. A \method of increasing the chemoprotective amount of Phase 2 enzymes in a mammal, comprising the step of administering an effective quantity of the cruciferous sprouts according to claim 1.
- 12. Crucifarous aprouts harvested prior to the 2leaf stage, wherein said sprouts have at least 200,000 units per gram fresh weight of Phase 2 enzyme-inducing potential when measured after 3-days of growth from seeds that produce said shrouts and non-toxic levels of indole glucosinolates and their breakdown products and goitrogenic hydroxybutenyl glucosinolates.
- 13. The cruciferous sprouts according to claim 12, wherein said sprouts ate a Brassies oleracea selected from the group of varieties consisting of acephala, alboglabra, botrytis, costata/ gemmifera, gongylodes, italica, medullosa, palmifolia, V ramosa, sabauda, sabellica, and selensia.
- 14. The cruciferous springs according to claim 13, wherein said sprouts are a Brassica oleracea variety italica.
- 15. The cruciferous sprouts according to claim 13, wherein said sprouts are a Brassica oleracea variety botrytis.
- 16. The cruciferous sprouts\according to claim 15, wherein said sprouts are a Brassica oleracea variety botrytis subvariety cauliflora.
- 17. A non-toxic solvent extract of the cruciferous sprouts according to claim 12.

CANDE ETEROSED

- The non-toxic solvent extract according to claim 17, wherein said solvent is water.
- 19. The non-toxic solvent extract according to claim 18, further comprising a cruciferous vegetable comprising an active myrosinase enzyme.
- 20. The non-toxid solvent extract according to claim 19, wherein said cruciferous vegetable is of the genus Raphanus.
- 21. A method of preparing a food product rich in glucosinolates, comprising germinating cruciferous seeds, with the exception of cabbage, cress, mustard and radish seeds, and harvesting sprouts prior to the 2-leaf stage to form a food product comprising a plurality of sprouts.
- 22. The method according to claim 21, wherein said sprouts contain non-toxic levels of indele glucosinolates their breakdown producis and N goitrogenic hydroxybutenyl glucosinolates.
- 23. The method according to claim-21, wherein said seeds are a Brassica oleracea selected from the group of varieties consisting of acephala, alhoglabra, botrytis, costata, gemnifera, gongylodes, italica, medullosa, palmifolia, ramosa, sabauda, sabellica, and selensia.
- 24. The method according to claim 23, wherein said seeds are Brassica oleracea variety italica.
- 25. The method according to claim 2\$, wherein said seeds are Brassica oleracea variety botratis.
- 26. The method according to claim 25, wherein said seeds are Brassica oleracea variety botrytis subvariety cauliflora.

CAMMIT ETBAGGE

- 27. A food product rich in glucosinolates made by the process according to claim 21.
- 28. A method of preparing a food product, comprising extracting glucosinolates and isothiocyanates from cruciferous sprouts according to claim 1 with a non-toxic solvent, removing the extracted sprouts from said solvent, and recovering the extracted glucosinolates and isothiocyanates.
- 29. A method of\preparing a food product according to claim 28, wherein \active myrosinase enzyme is mixed with said cruciferous sprouts, or said extracted glucosinolates and isothiocyanates, or both said cruciferous sprouts or said extract.
- 30. A method of preparing a food product rich in glucosinolates, comprising\germinating cruciferous seeds that produce sprouts having at least 200,000 units per gram fresh weight of Phase 2 enzyme-inducing potential when measured after 3-days of growth/and which contain non-toxic levels of indole \glucosinglates and their breakdown products and goltrogenic hydroxybutenyl glucosinolates, and harvesting sprouts prior to the 2-leaf stage to form a food product comprising a plurality of sprouts.
- 31. The method according to dlaim 30, wherein said seeds are a Brassica oleracea selected from the group of varieties consisting of acephala, alboglabra, botrytis, costata, gemmifera, gongylodes, Atalica, medullosa, palmifolia, ramosa, sabauda, sabellida, and selensia.
- 32. The method according to claim 31, wherein said seeds are Brassica oleracea variety italica.
- 33. The method according to claim \$1, wherein said seeds are Brassica oleracea variety botrytis.

- 34. The method according to claim 33, wherein said seeds are Brassica oleracea variety botrytis subvariety cauliflora.
- 35. A food product rich in glucosinolates, made by the process according to claim 30.
- 36. A method/of preparing a food product, comprising introducing cruciferous seeds, wherein said seeds produce sprouts having at \least 200,000 units per gram fresh weight of Phase 2 entyme-inducing potential when measured after 3-days of growth and non-toxic levels of indole glucosinolates and their breakdown products and goitrogenic hydroxybutenyl glucosinolates, into another edible ingredient.
- 37. A method of preparing a food product, comprising extracting glucosinolates and isothiocyanates with a nontoxic solvent and isothiocyanantes from cruciferous seeds, sprouts, plants or plant perts/wherein seeds that produce said sprouts, plant, or plant parts, have at least 200,000 units per gram fresh weight of Phase 2 enzyme-inducing potential when measured after 3-days of growth and wherein said seeds sproute, plants or plant parts have non-toxic levels of indole glucosinolates and their breakdown products and goitrogenic hydroxybutenyl glucosinolates, recdvering and glucosinclates and isothiocyanates.
- 38. A method of preparing a food product according to claim 37, wherein active myrosinase enzyme is mixed with said cruciferous seeds, sprouts or plants; or said extracted glucosinolates and isothiocyanates; or both said cruciferous seeds, sprouts or plants and said extract.
- 39. A method of reducing the level of carcinogens in a mammal, comprising administering to a mammal an

effective \amount of cruciferous sprouts, with the exception of cabbage, cress, mustard and radish sprouts.

- 40. A method of reducing the level of carcinogens in a mammal, comprising administering to a mammal an effective amount of cruciferous sprouts having at least 200,000 units per gram fresh weight of Phase 2 enzymeinducing potential when measured after 3-days of growth from seeds that produce said sprouts and non-toxic levels of indole glucosin plates and their breakdown products and goitrogenic hydroxybutenyl glucosinolates.
- 41. A method of extracting glucosinolates and isothiocyanates from plant tissue comprising the steps of homogenizing said plant tissue in an excess of a mixture of dimethyl sulfoxide, acetonitrile and dimethylformamide at a temperature sufficient to inactivate myrosinase enzyme activity.
- 42. A food product comprising cruciferous sprouts, with the exception of cabbage, cress, mustard and radish sprouts, harvested prior to the 2-leaf stage, cruciferous seeds; extracts of said | sprouts or seeds; or any combination of said sprouts seeds or extracts.
- 43. A method of increasing the chemoprotective amount of Phase 2 enzymes in a mammal, comprising the step of administering an effective quantity of the food product according to claim 42.
- 44. A food product compristing cruciferous sprouts harvested prior to the 2-leaf stage, wherein said sprouts have at least 200,000 units per gram fresh weight of Phase 2 enzyme-inducing potential when measured after 3days of growth from seeds that produce said sprouts and non-toxic levels of indole glucosimplate and goitrogenic hydroxybutenyl glucosinolates; /cruciferous extracts of said sprouts or seeds; or any combination of said sprouts, seeds or extracts.

- 45. A method of increasing the chemoprotective amount of Phase \2 enzymes in a mammal, comprising the step of administering an effective quantity of the food product according to claim 44.
- 46. Cruciferous sprofits harvested prior to the 2leaf stage, wherein the ratio of monofunctional to bifunctional inducers at least 20 to 1.
- 47. A food product supplemented with a purified or partially purified glucosinolate.

Zehder Erezesso

#### ABSTRACT OF THE DISCLOSURE

Vegetable sources of cancer chemoprotective agents have been identified which are extraordinarily rich in glucosinolates, metabolic precursors of isothiocyanates. The vegetable sources are used to provide a dietary means of reducing the level of carcinogens in mammals.

COTIONT BENEVERSO

5